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Inhibition of NO Synthase Activity in Nervous Tissue Leads to Decreased Motor Activity in the Rat

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Summary

The nitric oxide/cGMP system has been shown to play a crucial role in the mechanism of learning and memory. The aim of the present study was to investigate whether the inhibition of NO synthase in brain regions leads to alterations of spontaneous behavior in rats. Male Wistar rats were treated with NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) at the dose of 40 mg/kg/day. After 4 weeks of L-NAME treatment, NO synthase activity was significantly decreased by 75 % in the cerebellum, by 71 % in the cerebral cortex and by 72 % in the thoracic spinal cord. Decreased NO synthase activity in the nervous tissue was associated with decreased motor horizontal and vertical activities as well as by lowered frequency of sniffing, cleaning and defecation. It is concluded that the inhibition of NO synthase activity has a suppressive effect on spontaneous behavior of rats.

Key words

Nitric oxide • L-NAME • Nervous tissue • Spontaneous behavior • Habituation tasks

Introduction

Glutamate receptors and the nitric oxide (NO) system have been implicated in a large variety of pathophysiological conditions. Studies in different brain regions showed that glutamate receptors and the NO/cGMP system play a crucial role in the mechanism of learning and memory (Coan *et al.* 1987, Bohme *et al.* 1991, Schuman and Madison, 1991, Mysliveček *et al.* 1994). It has been demonstrated that the inhibition of NO synthase impaired spatial learning in rats, the conditioned eye-blink in rabbits (Chapman *et al.* 1992), radial maze learning (Bohme *et al.* 1993) place navigation learning in a water maze (Estal *et al.* 1992, Mogensen *et al.* 1995), working memory in a three-panel runway (Ohno *et al.* 1993) and taste avoidance of pecking in chicks (Hölscher and Rose 1993). On the other hand, NO synthase inhibition does not prevent the formation of olfactory memory in mice (Brennan and Kishimoto, 1993) and has no effect on the acquisition of the two-way active avoidance task (Prickaerts *et al.* 1998). While the effect of NO synthase inhibition on memory and learning has

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been studied extensively, only a few reports have investigated the effect of NO on spontaneous behavior with contradictory results in adult rats and rat pups (Yamada *et al.* 1995, Mysliveček *et al.* 1996, Wortwein *et al.* 1997).

The aim of the present study was to investigate whether long-term inhibition of NO synthase in brain regions by oral administration of N^G-nitro-L-arginine methyl ester (L-NAME) is associated with alterations of spontaneous behavior in adult rats.

Material and Methods

Animals and treatment

Male Wistar highly excitable rats (14 weeks old) were randomly divided into two groups (each n=8). The first group served as controls. In the second group, L-NAME (Sigma Chemical Co, Germany) was given in the dose of 40 mg/kg/day. The substance was given in drinking water for four weeks. Systolic blood pressure and heart rate (HR) were measured by the non-invasive method of tail-cuff plethysmography every day. Habituation tasks were performed at the end of every week. After 4 weeks, the animals were sacrificed and the homogenates of the cerebellum, cerebral cortex and thoracic spinal cord were prepared.

The animals were kept under controlled conditions at 22±2 °C and 55±5 % relative humidity and fed a regular pellet diet *ad libitum*. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No 8523, revised 1985).

Assay of NO synthase activity

The incubation buffer contained 25 mM HEPES, pH 7.4, 2 mM NADPH, 2.5 mM $CaCl_2$, 2 mM EDTA, 2 mM dithiotreitol, 20 μM L-arginine and 6.2 kBq of radioactive [2,3,4,5 - ^{3}H]-L-arginine hydrochloride. 50 μl of 10 % tissue homogenate was mixed with 50 μl of buffer and incubated for 15 min at 37 °C. The reaction was stopped by addition of 20 μl 1 M $HClO_4$ and the mixture was neutralized by 10 μl 2 M KOH. The control sample was treated in the same way but the homogenate was applied after adding $HClO_4$. After centrifugation, 50 μl of the supernatant with a standard solution containing an amino acid mixture of L-arginine, L-ornithine and L-citrulline was transferred onto chromatographic paper Whatman 4. The mixture was separated by electrophoresis in a pyridine-acetate buffer, pH 4.8, at a potential gradient of 25 V/cm.

After staining the amino acids with ninhydrin, the samples were excised in the area of L-citrulline and L-arginine and the conversion of L-arginine to L-citrulline was measured on a scintillation spectrophotometer.

Habituation tasks

The animals were tested in an exploratory box (developed at the Department of Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic) by the open-field test method at the end of every week. The animal was placed in the center of the exploratory box 80x60x40 cm in size with the floor divided into 8 squares. The horizontal and vertical motor activities, frequency of sniffing, cleaning and defecation were observed in a 20-min session in the same daytime period. On the basis of the habituation tasks, the animals were divided into high- and low-excitable groups before the experiment. Only the high-excitable animals (randomly divided into control and L-NAME groups) were used in the experiment.

Statistical analysis

The results are expressed as means ± S.E.M. For the analysis, one-way ANOVA and the Bonferroni test were used. The values were considered to differ significantly when the p value was less than 0.05.

Results

Cardiovascular parameters

After the first week of experiment, SPB was 126±5 mm Hg and HR was 380±6 beats/min in the control group In the group receiving 40 mg/kg/day of L-NAME, SPB increased by 35 % ($p<0.05$) and HR decreased by 24 % ($p<0.05$) as compared to the control group. The changes in SBP and HR persisted during the following three weeks. The body weight was not significantly affected in the L-NAME group (Table 1).

NO synthase activity

Different activity of NO synthase was shown in three different regions of the central nervous system. The highest NO synthase activity (520±20 pkat/g protein) was found in the cerebellum. Lower activity (185±9 pkat/g protein) was noted in the cerebral cortex and the lowest

one (72±3 pkat/g protein) was observed in the thoracic spinal cord. In the L-NAME group, NO synthase activity was inhibited by 75 % ($p<0.05$) in the cerebellum, by 71 % ($p<0.05$) in the cerebral cortex and by 72 % ($p<0.05$) in the thoracic spinal cord as compared to the control group (Fig. 1).

Table 1. Effect of 4-week L-NAME treatment (40 mg/kg/day) on systolic blood pressure (SBP), heart rate (HR) and body weight (BW).

	SBP (mm Hg)	HR (beats/min)	BW (g)
Controls	126±5	380±6	441±8
L-NAME	170±7*	288±9*	429±9

** $p<0.05$ as compared to the control group.*

Habituation tasks

Motor horizontal and vertical activity in the L-NAME group was significantly decreased ($p<0.05$) after the first and second weeks of L-NAME treatment. In the following two weeks, the tendency to decreased motor horizontal and vertical activity still persisted, but it was no longer significant as compared to the control group (Fig. 2).

Sniffing and defecation in the L-NAME group was significantly decreased ($p<0.05$) at all studied time periods as compared to the control group (Figs 3 and 5).

Cleaning in the L-NAME group was significantly reduced ($p<0.05$) in the second, third and fourth week of L-NAME treatment as compared to the control group (Fig. 4).

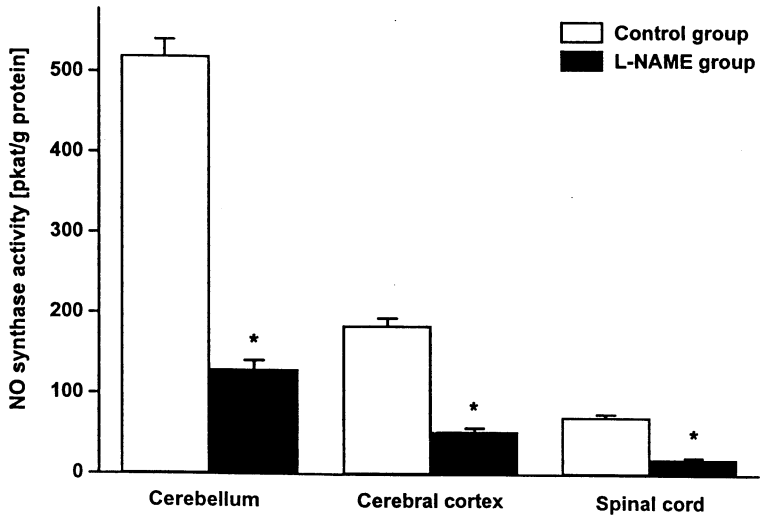

Fig. 1. *Effect of 4-week L-NAME treatment (40 mg/kg/day) on NO synthase activity in cerebellum, cerebral cortex and thoracic spinal cord. *$p<0.05$ as compared to the control group.*

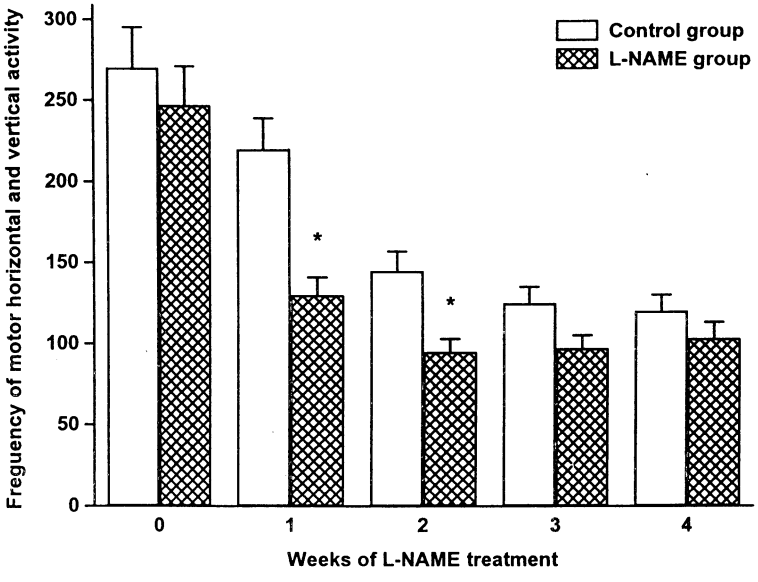

Fig. 2. *Effect of L-NAME treatment on motor horizontal and vertical activity of rats. *$p<0.05$ as compared to the control group.*

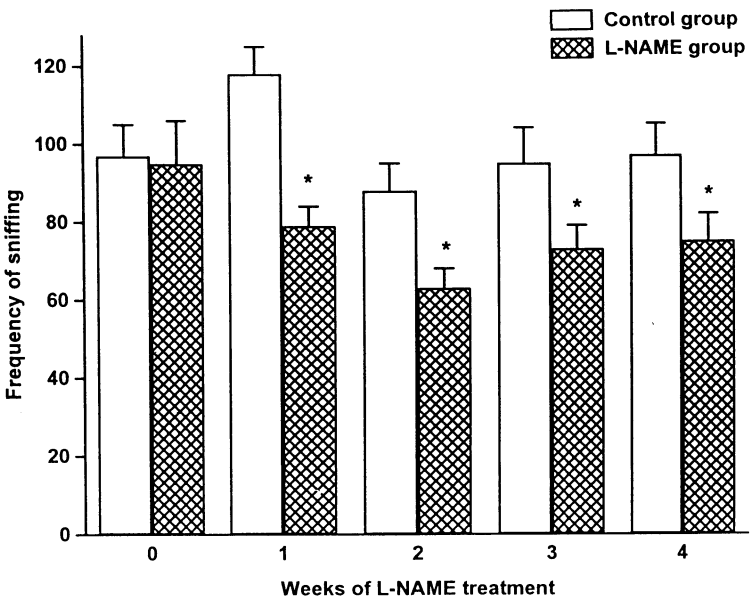

Fig. 3. *Effect of L-NAME treatment on frequency of sniffing of rats. *p<0.05 as compared to the control group.*

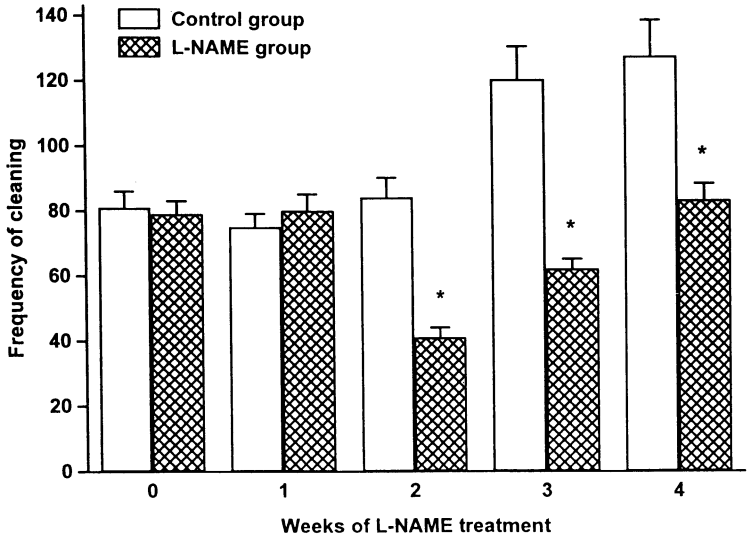

Fig. 4. *Effect of L-NAME treatment on frequency of defecation of rats. *p<0.05 as compared to the control group.*

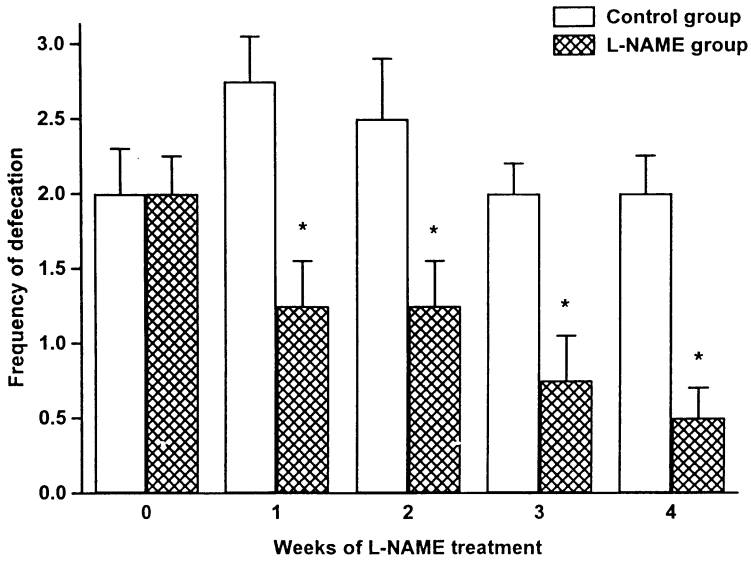

Fig. 5. *Effect of L-NAME treatment on frequency of cleaning of rats. *p<0.05 as compared to the control group.*

Discussion

The present study demonstrated that decreased NO synthase activity in the cerebellum, cerebral cortex and thoracic spinal cord was associated with decreased motor horizontal and vertical activities, decreased frequency of sniffing, cleaning and defecation.

The experiments showed considerable differences in NO synthase activity in various parts of the central nervous system of the rat. The highest activity was detected in the cerebellum, lower in the cerebral cortex and the lowest in the thoracic spinal cord. The high NO synthase activity in the cerebellum is in agreement with results described by other authors (Förstermann *et al.* 1990, Kidd *et al.* 1995). The relatively low activity in the cerebral cortex corresponded well with the histochemical study of Dawson *et al.* (1992), who reported that only 2 % of cerebral cortex neurons exhibited NO synthase activity. Barjavel and Bhargava (1995) also observed the highest NO synthase activity in the cerebellum, followed (in a decreasing order) by the midbrain, hypothalamus, cortex, striatum, pons-medulla, hippocampus and spinal cord. Evidence has been presented that L-NAME crosses the blood-brain barrier (Dwyer *et al.* 1991), and it was hence administered intravenously or intraperitoneally in many previous studies to inhibit NO synthase in the brain. We have shown that oral L-NAME treatment also led to NO synthase inhibition in the cerebellum, cerebral cortex and thoracic spinal cord with comparable inhibition (by about 73 %) in the studied brain regions. Using the same experimental model, NO synthase activity in the L-NAME treated group from our previous study was also found to be decreased in the left ventricle of the heart, aorta and kidney (Bernátová *et al.* 1996, Pecháňová *et al.* 1997). L-NAME, as well as many other NO synthase inhibitors, is strongly vasoactive and produces significant constriction in different parts of the vascular tree (Török and Gerová 1996). Systemic administration of L-NAME induces a marked increase in systolic blood pressure and a decrease in heart rate, as has also been confirmed in the present study. These hemodynamic changes are in agreement with the findings of several other authors (Ribeiro *et al.* 1992, Xie *et al.* 1996).

In our experiment, the inhibition of NO synthesis in the central nervous system had the suppressive effect on motor horizontal and vertical activities, on the frequency of sniffing, cleaning and defecation. Similarly, Jachimowicz *et al.* (1998) found decreased activity of animals in the "open field test" after L-NAME treatment. On the other hand, the NO synthase substrate – L-arginine – increased the psychomotor behavior of rats. The administration of another NO synthase inhibitor N^G-nitro-L-arginine impaired emotional habituation based on defecation scores and habituation of the vertical component, known to be mainly of emotional significance (Papa *et al.* 1994). In mice, L-NAME treatment did not produce any overt behavioral changes and failed to influence locomotor activity or the incidence of dipping, crossing, rearing or circling behavior assessed by a modified head-dipping board procedure. A high dose of L-NAME (600 mg/kg) reduced dipping behavior and locomotor activity suggesting a possible sedative effect (Moore *et al.* 1991). In contrast to these studies, the speed of habituation of locomotion in an open field in adult rats, which had been treated postnatally with NO synthase blocker, was found to be increased (Wortwein *et al.* 1997). Furthermore, L-NAME treatment appeared to increase the behavioral activity of rat pups in the open field (Prickaerts *et al.* 1998). These data indicate that the habituation to novelty triggers a cascade of neurochemical events also involving nitric oxide. The differences observed in habituation after the inhibition of NO synthesis between adult rats and rat pups may be caused by the higher NO synthase activity in pups than in adults, by different doses of NO synthase inhibitor and duration of treatment as well as by various time-periods of the studied habituation tasks.

Taken together, the decreased availability of NO in the brain regions leading to the suppression of habituation may also effect the emotional information process.

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